

Introduction: Biomarkers of Toxicity During Pregnancy

by Richard K. Miller*

Pregnancy brings a series of physiological and biochemical changes that transform a woman from a single functioning unit to an organism within an organism. During pregnancy, there are two sets of genomes interfacing through a unique organ called the placenta. Dynamic change is observed throughout pregnancy for the female of all mammalian species. When superimposed upon pregnancy, the exposure to a therapeutic or environmental agent produces multiple factors. Does the agent affect only the mother, only the conceptus, only the placenta, or all three? These unique interactions are based upon alterations during pregnancy and the appearance of two functioning units. Unique physiological and biochemical characteristics of pregnancy include the presence of two separate blood supplies; rapid and selective growth of specific cell types in the conceptus at particular stages of conception; and direct and indirect interactions among the mother, embryo/fetus, and placenta (1). Function of maternal organs, not to mention the embryonic/fetal organs, may be substantially different at specific stages of gestation. Thus, the challenge to the clinician and scientist is to assess pregnancy/developmental status and the impact of such xenobiotic exposure upon the conceptus.

To assess such impact, biomarkers have been used for generations. Clinical signs of amenorrhea, nausea, weight gain, the appearance of an embryonic heartbeat, and fetal movement have all been used for centuries. Such biomarkers have substantial limitations in sensitivity or the early documentation of pregnancy, e.g., fetal movement or quickening being greater at 16 weeks of gestation, or nonspecificity, e.g., amenorrhea, an infertility problem and not pregnancy. Certainly the development of specific hormone assays, such as measurement of human chorionic gonadotropin (hCG), has provided a greater specificity to detect pregnancy (2). The evolution of this hCG assay from a bioassay to a highly sensitive enzyme immunoassay has provided both the patient and the clinician the capability to detect a pregnancy before the clinical signs of a pregnancy are

noted, i.e., the absent menstrual period. Such a biomarker has been a tremendous asset to the care of both the pregnant patient and the infertile couple. A new terminology has developed surrounding such a biomarker and its early assessment: chemical pregnancy. In past years, many women would experience a late menstrual period and attribute it to an altered cycle; however, today, because of the sensitivity of these assays that enable early detection of pregnancy, many more pregnancies are being documented that fail early. The introduction of such a sensitive biomarker initially suggested that women today were being exposed to toxins that produced early pregnancy loss because of the tremendous increase in documented early pregnancy loss. However, what was actually observed was the efficiency of normal human reproduction, which had not been previously documented. Such documentation of early pregnancy loss in a population demonstrates how critical it is to use a biomarker appropriately for the assessment of normal function and for the assessment of potentially toxic influences on that function.

Thus, in relationship to pregnancy, many of the normal biomarkers of maternal function and development cannot be easily extrapolated to toxicity assessment. Obviously, the premier goal of the following papers is to assess the state-of-the-art for all biomarkers related to reproduction and development and to assess these biomarkers in relationship to selected adverse effects on either mother or conceptus for specific exposure.

Biomarkers related to pregnancy and development can be divided into two major categories: exposure or toxicokinetics, and effects or toxicodynamics (Table 1). Obviously, the objective for performing any of these assessments is to determine whether a therapeutic or environmental exposure has produced a detectable insult to mother or conceptus. The second objective is to determine if this insult can be detected or estimated sufficiently early to prevent or reverse the observed insult. For example, the detection of the birth defect spina bifida (open neural tube) is easily documented at birth. However, it is important to know that this defect is present before parturition so that the method of delivery may be altered. Ultrasonography has been most

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Table 1. Biomarkers associated with pregnancy.

Maternal	Epidermal growth factor
Serum/plasma	Folate
Urine	Immunoglobulin (IgG-Fc)
Feces	Low density lipoproteins
Hair	Opiates
Nails	Somatomedin
Tissue biopsy	Testosterone
Endometrium	Transcobalamin II
Cervical mucus	Transferrin
Follicular fluid	TCDD
Uterine washings	Binders and other proteins/tissue products
Fat	Transcobalamin I/II/III
Products of conception	α -Fetoprotein
Placental: Products of conception	Enzymes and carrier proteins
Chorionic villus biopsy (early)	DNA adducts
Amnion	Nutrient and xenobiotic metabolism
Chorion	Nutrient and xenobiotic transport
Umbilical cord	Karyotyping
Fetal blood/serum/plasma	Morphometry
Amniotic fluid	Histology
Embryo/fetus: Products of conception	Embryo and fetus:
Tissue analysis	Tissue-specific antigens
Blood/serum/plasma	CEA
Hair	α -Fetoprotein
Nails	Immunoglobulins
Urine	Physical examinations
Feces	Physical
Amniocentesis	Carcinogenesis
Amniotic fluids	Dysmorphology
Fetal cells	Apgar/Braseltton
Foreskin	Death
Maternal: Steroids	Incidence of pregnancy loss
Uterine prolactin	Growth retardation
Immune responses	Growth Factors
HLA	IGF
TLX	EGF
Histology	NGF
Vaginal epithelium	Physiology
Endometrium	Inborn errors of metabolism
Cervical mucus	Organ system specific
Toxic screens	Cardiovascular
Binding globulins and other proteins	Hepatic
Uterine blood flow changes	Renal
Pregnancy-associated carcinogenesis	Neural
(Trophoblastic neoplasia)	Others
Placental: Hormones	Assessment tools (current)
Human chorionic gonadotropin	Amniocentesis
Human placental lactogen	Chorionic villus biopsy
cACTH	Doppler blood flow
cFSG	Fetal blood sampling
cTSH	Fetal heart rate monitoring
PAPP-A	Fetoscopy
Interferon	Magnetic resonance imaging
Interleukin I	Products of conception
Estradiol	Ultrasound real time
Estriol	Intact organ chemiluminescence
Progesterone	Incidence of pregnancy loss
Receptors	Fluid and tissue analysis
β -adrenergic	Molecular genetic/DNA probes
Glucocorticoid	<i>In vitro</i> comparisons

helpful in documenting the appearance of spina bifida *in utero*. However, there are limitations to ultrasonography in detecting the defect during the first trimester. The introduction of maternal serum and amniotic fluid α -fetoproteins as indicators of neural tube defects has

been most helpful in the early detection of such a defect. Yet, the goal of this type of investigation is the development of a biomarker which establishes that the patient/conceptus is at risk for having such a defect before the defect is apparent. If risk detection is possible, then

modification of exposure and possible corrective therapy for the exposed patient or conceptus may be possible to eliminate such a problem.

Such goals of reduced exposure or identification of susceptible individuals, whether mother or conceptus, require new directions for identifying biomarkers, but even more importantly, these goals require an extremely judicious validation process for determining the usefulness of each biomarker. To assess these goals for perinatal toxicology and the potential for achieving them, internationally recognized experts in the field of reproduction and toxicology have been asked to discuss biomarkers of both effect and exposure, applying the latest in technological advances and criteria of validation to the issues of human risk analysis.

In addition to the assessment of hCG, perhaps the premier biochemical marker for assessing the risk of birth defects in the human is the measurement of α -fetoprotein (AFP). At the NAS Symposium, David Erickson, from the Center for Disease Control in Atlanta, Georgia, reviewed the usefulness of AFP in maternal serum and amniotic fluid as a biomarker of risk. It was stressed that both the sensitivity and specificity of a test are essential to its predictive value, where sensitivity is the probability that AFP will indicate the neural tube defect given the actual presence of the disease. A perfect biomarker in terms of sensitivity would not give false negatives. Specificity refers to the ability of AFP to indicate the absence of neural tube defects when there is no disease present. Thus, the use of a biomarker, e.g., maternal serum AFP, has limited usefulness as the only tool for identifying neural tube defects because of the sensitivity levels; however, maternal serum AFP is a successful screen in combination with other clinical tools (e.g., ultrasonography, chromosomal screening, or acetylcholinesterase activity), and it has potential usefulness as a screen for associations between valproate therapy or insulin-dependent diabetes mellitus and neural tube defects. These biomarkers are the types required for screening programs which reduce misidentification of an individual who is at risk for a neural tube defect from a large and heterogeneous population.

Among the most exciting areas of investigation today, two relate to the early, shared signals between the endometrium and trophoblast that reflect the capability of the embryo to implant and develop. Many of these issues have been further enhanced by the international success of the *in vitro* fertilization programs. Thus, once fertilization has occurred, the major issues are related to how the implantation process may be affected and limited by either maternal or fetal factors. Such factors may be the tissue development, the specific signals produced, or the specific antigens carried on the trophoblast, in addition to the obvious chromosomal issues of perimplantation lethality. Stanley Glasser has assessed these signals from both the trophoblast and endometrium as potential biomarkers for pregnancy outcome and toxic influence (3). W. Page Faulk has documented the importance of cell-surface antigens on the tropho-

blast to the capability of the conceptus to survive implantation (4). The importance of HLA antigens is reviewed as is the most exciting work on a TLX antigen that has been associated with patients who are primary spontaneous aborters. Such biomarkers for determining the causes of failed pregnancy are essential to our understanding of how new biomarkers may be used to eliminate nontherapeutic/environmental causes of failed pregnancies.

As gestation progresses, many other interventions for assessing development have been used to evaluate the impact of xenobiotics not only on the fetus but also on the mother. The assessment of fetal blood flow via Doppler analysis and cardiac function via echocardiography have been reviewed by Lawrence Longo (5). The ability to assess the effects of hypoxia itself, as well as the effects of xenobiotic exposure, is being identified as essential areas of investigations for all perinatologists. Obviously, the alterations of fetal chest wall movements due to a single cocktail or to a cigarette illustrate the capability of identifying effects on the fetus. Of equal importance is establishing the importance of such changes to the immediate function of the fetus and the long-term development of the child.

Many assessments noted only identify the consequences of the therapeutic or environmental exposure. Often the only intervention is delivery, if that is possible. As stated above, the major objective of the use of biomarkers is to identify susceptible populations and either prevent or modify the outcome in a positive manner. Thus, innovative work reviewed by Richard Everson implicated both the presence of an agent and its ability to interact with cellular constituents in the conceptus (6). In particular, studies have used placentae from women who have smoked or were exposed to specific polycyclic aromatic hydrocarbons. In these tissues, specific adducts to cellular DNA have been identified. Currently, the patterns of DNA adducts formed from many of these compounds appear to have a blot pattern that is specific for the compound. Thus, the ability to detect specific compounds reacting with fetal tissue could perhaps be initially assessed by sampling chorionic villus tissue. Such studies raise further interest in the ability of other fetal tissues, e.g., amniotic fluid cells, to produce a similar DNA-adduct profile.

With the formation a specifically identifiable fingerprint DNA adduct for a compound, it may be possible to assess not only affect but also exposure, which is the second class of biomarkers. Perhaps the premier example of estimating a dose-response relationship for a human teratogen has uniquely occurred because of the exposure of a rural population of families in Iraq to grain treated with the fungicide methylmercury. Thomas Clarkson and his team have intensively evaluated this population for the past 15 years (7). Clarkson reports methods whereby one can assess the actual exposure to methylmercury prior to, throughout, and well into the postnatal period by assessing the content of mercury in a single strand of hair. This ability to assess actual exposure has been invaluable. Such assessments have pro-

vided the opportunity to determine the actual timing and amount of exposure *in utero* and postnatally. Dose-response relationships have been documented. Thus, it is possible that for selected metals, analysis of hair and correlation with other tissues and body fluids may be essential to establishing risk even before conception.

To establish potential risk, estimating exposure to methylmercury can be critical. To establish such exposure to other compounds by using a biomarker is often extremely difficult. Pharmacokinetic assessments are often measurements of only maternal blood or urine, and extrapolations are performed to project levels in the conceptus or measures of tissue content based upon placental tissue or hair/fat analyses at delivery (8). Unfortunately, the inaccessibility of the conceptus has presented problems for such exposure monitoring during pregnancy. However, Richard K. Miller reviews some of the most recent advances in perinatology utilizing magnetic resonance imaging (MRI) as a tool for evaluating the conceptus. In addition, *in vitro* models for assessing the placental passage of therapeutic and environmental agents during a 24-hr period are evaluated to establish the mechanism by which the human fetus can be exposed to nutrients, drugs, and environmental chemicals (8,9). The use of magnetic resonance imaging to identify physical defects is but one possibility. To assess individual organ function at a biochemical level is the exciting potential for such spectroscopy. For example, it has been possible under selective conditions to measure phosphorus as ATP, ADP, AMP, and phosphocreatine. Such capabilities for the conceptus may identify specific organ response without awaiting a generalized response of the conceptus. To actually measure the distribution of specific compounds is now possible. However, limited studies have been performed in the pregnant woman to date because of a lack of information concerning the effects of MRI itself on the pregnant

woman and the conceptus. Thus, for toxicokinetic purposes, dual perfusion of the isolated human placental cotelydon has provided the opportunity to assess extremely toxic agents, TCDD or cadmium, in the human placenta without risk to either mother or conceptus.

Thus, the following chapters represent many state-of-the-art applications which should stimulate the reader's imagination to explore further the issues of the use of biomarkers of toxicity during pregnancy, whether expressed as effect or exposure markers.

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